

SOP: PP054.1

Date modified: 7-25-16 ASimpson

Establishment of frozen glycerol stocks of *Mycobacterium smegmatis*, small scale

Material and Reagents:

1. *M. smegmatis*, frozen seed stock located in micro contract -80
2. Pipet and sterile filter tips
3. Inoculation loops
4. Incubator with stir plate
5. Plate scrapers
6. 250mL plastic E flask and stir bar
7. Bunsen burner
8. 40x 1.7 ml cryovials
9. 20% Glycerol in GAS or 7H9/OADC/tween media (note 1)
10. 10 ml sterile, plastic pipet
11. Electronic pipeter
12. Biosafety hood

Protocol:

1. _____ Inoculate plates with 200µL seed stock or working stock, place in a Ziploc bag, and grow in 37°C incubator until confluent (1-2 weeks) (note 1).
2. _____ Collect all your materials and plates into the biosafety hood. Scrape plate into 250mL E flask (note 2).
3. _____ Autoclave trash and clean hood. Incubate E flask at 37°C for ~4 days.
4. _____ Collect all your materials and E flask with suspended cells into the biosafety hood.
5. _____ Flame E flask top and aseptically aliquot 1mL/cryovial. Label cryovials (note 3).
6. _____ Take a loop of the last vial and streak on nutrient ager to confirm culture is uncontaminated.
7. _____ Place nutrient ager in a Ziploc bag at 37°C for 1 week then discard if no growth. Store cryovials in contract -80 in micro. Label exterior of cryovial box (note 3).
8. _____ Add Vesphene to E flasks and autoclave trash. Save stir bars. Clean hood.

Notes:

1. A seed stock is often the oldest batch of stocks derived from an original source (history may be difficult to track on older strains). When possible, seed stocks are made from one colony of an original source streaked for isolation. Colony is then streaked and grown till confluent. Working stocks are derived from seed stocks.
2. Scrape 7H11 plate into a plastic 250mL E flask containing 50mL 20% glycerol in GAS or 7H9/OADC/tween media. Do several flasks if more than 50mL needed. Grow ~4 days at 37°C on stir plate with stir bar or until media shows good growth. Stir plate is better than shaker table due to the highly flocculent nature of smeg.
3. Label contents: Genus species, strain, lot # (year.strain.month.day.pass#), volume, media, initials of technician, date aliquoted, any other useful info, for research only.